COUPLING THE DEVELOPMENTAL PROGRAMS OF TEETH AND TASTE BUDS IN MALAWI CICHLIDS

A Thesis
Presented to
The Academic Faculty

by

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In Partial Fulfillment
of the Requirements for the Degree
B.S. in Biology with Research Option in the
School of Biology

Georgia Institute of Technology
May 2009

COUPLING THE DEVELOPMENTAL PROGRAMS OF TEETH AND TASTE BUDS IN MALAWI CICHLIDS

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Date Approved: Friday, May 1, 2009

ACKNOWLEDGEMENTS

I wish to thank Dr. Todd Streelman, Dr. Gareth Fraser, and all the laboratory colleagues for their guidance and encouragement.

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LIST OF SYMBOLS AND ABBREVIATIONS

AP	Alkaline Phosphatase
β-cat	beta-catenin (activates transcription in Wnt Signaling Pathway)
BMP	Bone morphogenetic protein
BCIP	5-Bromo-4-chloro-3-indolyl-phosphate
BLAST	Basic Local Alignment Search Tool
C	Celsius
DEC1	Deleted-in-esophageal cancer 1, also stra13/sharp2 (transcription factor)
DEPC	Diethyl pryocarbonate
Dlx	*distal-less* like homeobox (homeobox transcription factor)
DNA	Deoxyribonucleic Acid
Dpf	Days post-fertilization
EDA	Ectodermal dysplasia-A protein (tumor necrosis family member)
EDAR	Receptor protein for EDA
FGF	Fibroblast growth factor (peptide growth factor)
MABT	maleic acid buffer with Tween 20
mRNA	messenger ribonucleic acid
NCBI	National Center for Biotechnology Information
Notch	Notch transmembrane receptor protein for Delta ligand
NTB	4-nitroblue tetrazolium chloride
NTMT	NaCl - Tris HCl - $MgCl_2$ buffer with Tween 20
Pax	Paired-like homeobox (homeobox transcription factor)

PBS	Phosphate buffered saline
PBST	Phosphate buffer saline with Tween 20
PFA	Paraformaldehyde
Ptc	Patched (repressor subunit in Hh-receptor complex)
Pitx2	Pituitary homebox 2 (homebox transcription factor)
RNA	Ribonucleic acid
Shh	Sonic Hedgehog (signaling molecule)
Tbx	T-box 1 (transcription factor)
Tris-HCl	Tris(hydroxymethyl)aminomethane buffer with hydrochloric acid
TST	NaCl - Tris HCl – DEPC H_20 with Tween 20
µm	micrometer (10^{-6} meter)
Wnt	Wingless (signaling molecule)

SUMMARY

Epithelial tissue of vertebrate organisms serves as the interface between them and the immediate environment with which they interact. Transformation of this outer tissue layer generates specialized structures that can allow organisms to make enhanced or entirely new interactions with its ecological niche. With only two different modes of development, the epithelium can derive such structures as hairs, feathers, scales, horns, glands, teeth, and taste buds. This study, however, focuses on two structures generated from the oropharyngeal cavity epithelium: teeth and taste buds. Using cichlids from Lake Malawi in eastern Africa as a model, this study seeks to show the co-evolutionary relationship that likely exists between teeth and taste buds. Based on the observations that both teeth and taste buds are derived from the epithelium, are colocalized sensory organs within the oropharyngeal cavity, have very similar structures in early development, and share certain patterns in gene expression, we hypothesize that the gene networks governing tooth and taste bud development are similar. Through comparative morphology and molecular developmental biology, this study shows that both teeth and taste buds share similarities of gene expression in both spatial and temporal patterns. As with most observations in evolutionary biology, the co-evolutionary history of teeth and taste buds cannot be proven, rather it can only be supported by sound evidence. However, future studies could further support and strengthen this theory by demonstrating possible correlations of gene function in the development of teeth and taste buds using functional genomics or even by uncovering unforeseen numerical relationships between teeth and taste buds.

CHAPTER 1

INTRODUCTION

Epithelia serve as the interface between organisms and their environment. The first point of contact a vertebrate ever makes with its niche is with its integumentary structures. With such a pivotal role in an organism's life, it is not surprising that significant selective pressure has acted upon the ability of the epithelium to maximize survival and reproduction. Advantageous mutations originating within the developing epithelium were rewarded with positive selection as they would have enabled species to occupy new niches. With more niches being filled, an impressive adaptive diversification of epithelium derived structures has radiated over evolutionary time. These structures constitute a lengthy array of specialized appendages such as hairs, feathers, scales, horns, beaks, claws, nails, teeth, taste buds, and mammary, sweat, and salivary glands. Data from comparative morphology, developmental biology, and functional genomics would provide new insight into the patterns and mechanisms of evolutionary modification in vertebrate epithelial structures. This study, however, examines two critical integumentary organs of the oropharyngeal epithelium: teeth and taste buds. A comparison of the developmental and genetic patterns of teeth and taste buds in Malawi cichlids can shed new light onto mechanisms by which shared gene pathway networks govern the initation and differentiation of very similar early epithelial thickenings into very different specialized organs.

Developmental Theme in Epithelial Structures

Although hairs, feathers, teeth, taste buds and all of the specialized integumentary organs may be morphologically and functionally different, they are not developmentally disparate as the mechanisms of their development follow a common theme. Epithelial appendages, which are merely elaborate topological transformations, share four

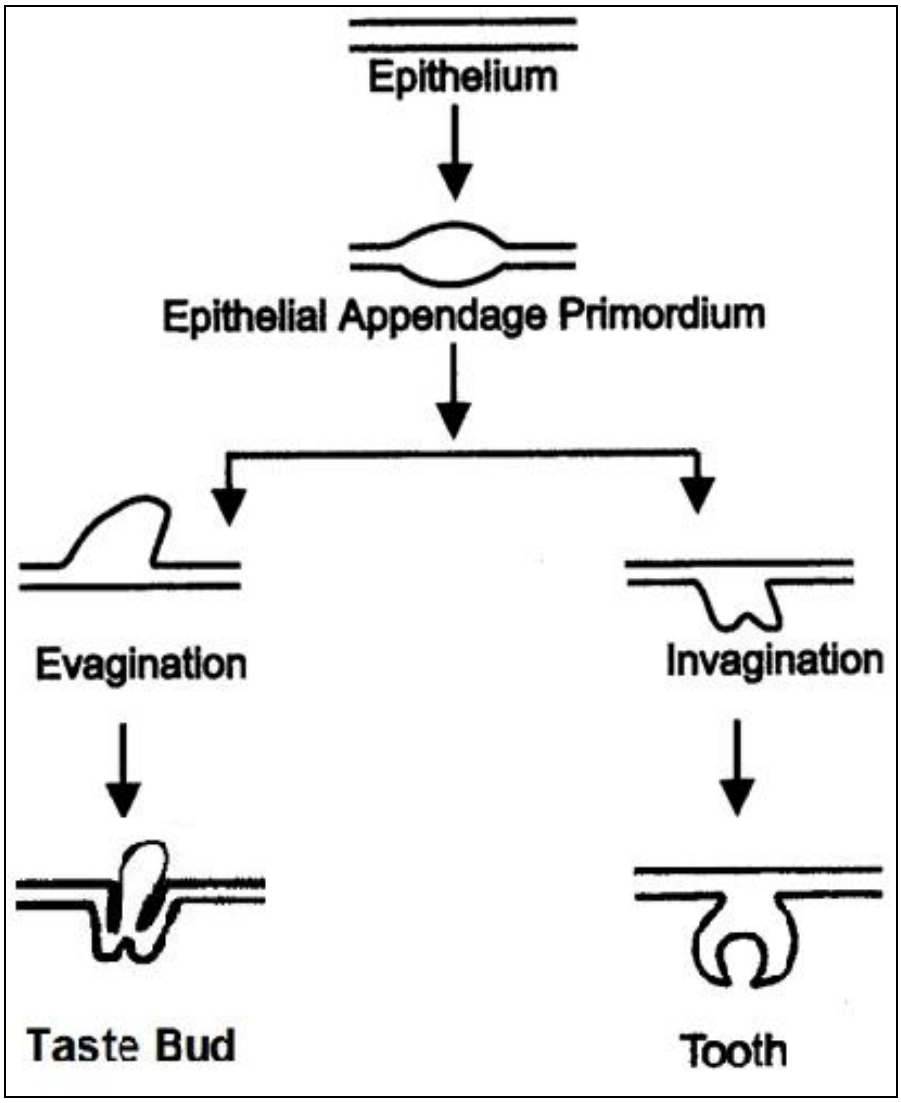

Figure 1. Two Modes of Epithelial Transformation. Once the epithelium becomes initiated to form thickened epithelial primordia, one of two different paths of morphogenesis is followed. On the left side of the figure is the path followed by taste buds in which the epithelium grows outward away from the mesenchyme. The right side of the figure illustrates the transformation of teeth buds whereby the epithelium protrudes progressively inward into the mesenchymal tissue.

developmental stages: initiation, morphogenesis, differentiation, and cycling. These patterns epithelial transformation are illustrated in Figure 1 that has been modified form (Chuong *et al., 2000).* It is currently accepted, however, that during the morphogenesis stage, two different modes of transformation occur in the epithelium. The first is the method by which the induced epithelial tissue begins an early dialogue of signaling with the underlying mesenchyme and advances to invaginate into the substratal tissue, as is the case for teeth and glands. The second type of epithelial transformation is just the opposite, whereby the induced epithelium evaginates above the surrounding epithelial layer, without a direct interaction involving the underlying tissues, to form such structures as taste buds, hairs, and feathers (Chuong *et al., 2000).*

Early Similarities between Teeth and Taste Buds

Early in embryonic development, teeth and taste buds appear very similarly histologically as they both exist as thickened epithelial primordia in their respective induction stages. The first teeth, however, always initiate earlier than the first taste buds in Malawi cichlids. Also, the two structures express many of the same genes. Specifically, in situ hybridization techniques have shown that both early teeth and taste buds express genes involved in the Shh, Wnt, Fgf, and BMP pathways. As development progresses from the induction to morphogenesis stages, teeth and taste buds no longer appear the same, as shown in Figure 2.

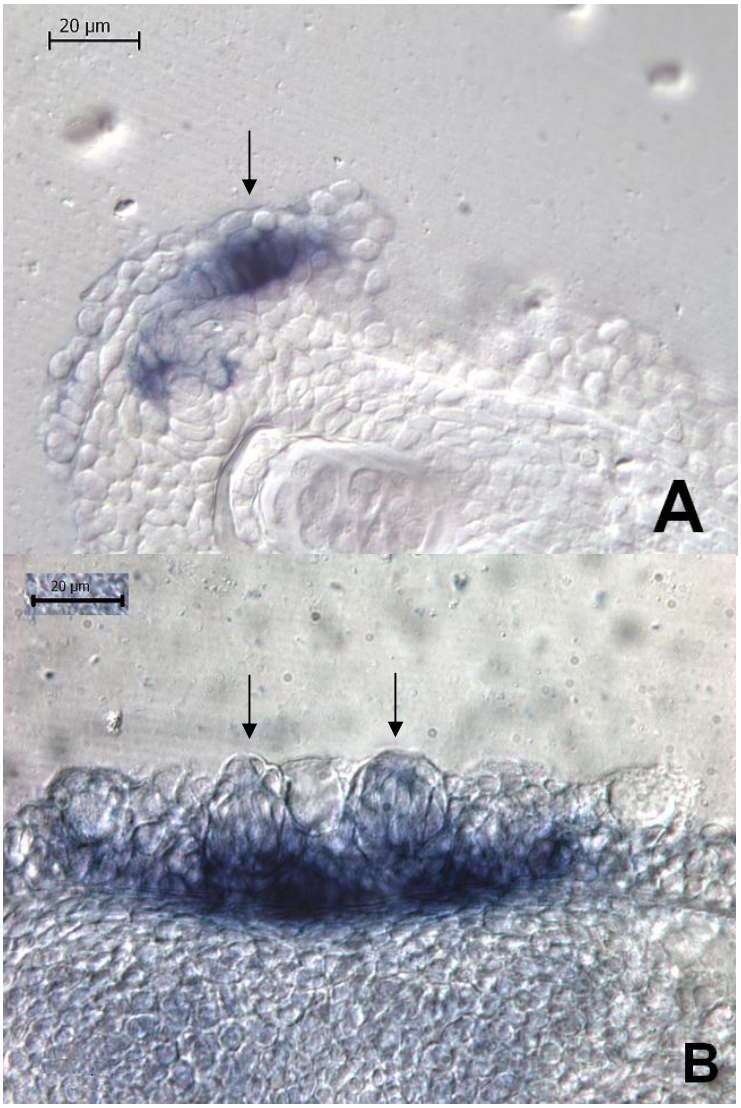

Figure 2. Structural Similarity of Initiated Tooth and Taste Bud. Shh, a positive marker for tooth buds and taste buds, is shown strongly expressed in a developing (A) tooth bud of *L. fuelleborni* at 6dpf and in (B) taste buds of *L. fuelleborni* at 14dpf. Note the nearly indistinguishable structures of a tooth bud and taste bud in their epithelial placode stage.

Tooth Development

Once thickened dental epithelium (in the oral jaws it is termed the dental lamina) receives specific signals from the subjacent mesenchyme, the induced epithelium activates networks of gene pathways that induce cell proliferation in the direction of the mesenchymal tissue (Kettunen et al, 2000, Sarkar et al, 1999). Once this occurs, the morphogenesis stage is well underway as the invaginated epithelium then becomes a tooth bud and the surrounding the tissue condenses into dental mesenchyme. The bud will continue to grow and eventually form a primary enamel knot, which serves as a signaling center for further tooth development and the formation of cusps (the number of cusps formed is dependent on the species and dental areas within a species) (Vaahtokari *et al*. *1996*). Next is the differentiation stage of tooth development whereby the tooth bud's thickened epithelium takes on the shape of a cap. The "cap" is often referred to as the enamel organ as it becomes surrounded by the dental follicle while confining the dental papilla. The subsequent bell stage is marked by further cell differentiation, such as the inner dental epithelium that becomes ameloblasts, and the disintegration of the dental lamina. The last stage before eruption is the crown stage whereby the signature hard tissues enamel and dentin become mineralized by ameloblasts and odontoblasts, respectively, starting with the cusps (Peters *et al*., *1999*).

Taste Bud Development

Taste buds begin their development in the same manner as teeth do in the form of epithelial primordia. Since this epithelium is receiving no signal from the mesenchyme, the intrinsic genetic pathway for taste bud development will be activated and restricted to the epithelium (Barlow *et al., 1997).* These pathways, which include Shh, Wnt, and Fgf, signal for morphogenetic cell proliferation away from the underlying mesenchyme and above the surrounding epithelium. Soon after the induced epithelium has evaginated above the level of the rest of the epithelial tissue, the taste bud will enter the

differentiation stage of its development (Northcutt *et al*., *1998*). Research has shown, in *Danio rerio* (Zebrafish) at least, that the dark cells of taste buds differentiate before light cells (Hansen *et al., 2002*). The dark and light cells of taste buds are so named because of their electron density. The fully developed taste bud takes on the appearance of a pear in which only small, chemosensory microvilli are exposed to the superficial environment in the oropharyngeal cavity while the body of the taste bud proper remains buried within the epithelium (Boudriot *et al., 2001)*. At the base of the taste bud is the nerve fiber plexus where the taste bud is innervated (Torrey, *1940*). The specific mechanisms describing the development of innervations is still only partially understood (Northcutt, *2004*). Many studies have shown that the taste buds of fish are connected to the brain stem the facial (VII) nerve, glossopharyngeal(IX), and vagus (X) nerves (Fishelson et al, *2004*).

Unique Characteristic of Taste Buds

A unique feature of taste buds is that they are capable of developing not only without mesenchymal signal induction, but also in the absence of the mesenchyme itself. A clever explantation experiment involving salamander embryos was conducted in which the presumptive oropharyngeal cavity was removed and placed into a culture medium. After nine to twelve days, well-differentiated taste buds had developed in the explants. These results demonstrate that the ability to develop taste buds is an intrinsic feature of the oropharyngeal epithelium and do not require induction by innervation (Barlow *et al, 1997*). Additionally, similar experiments demonstrated that although taste bud primordia may not require nerve fibers for induction, subsequent differentiation and maintenance may require them (Northcutt, *2004*).

Experimental Plan

The epithelially intrinsic gene networks directing taste bud development has not yet been described. However, much research has shown what genes are involved in odontogenesis. Researchers have demonstrated a core dental regulatory network common to all vertebrates. During the initiation stage, core gene markers of the dental epithelium are shh, pitx2, bmp2, edar, and to a lesser extent, bmp4, dlx2, and eda. Simultaneously, the subjacent mesenchyme releases several signaling molecules that activate tooth development including bmp2, bmp4, dlx2, and eda. Once activated, the epithelium expresses β-cat, fgf3, fgf10, and notch2 for initiation of the dentition (Fraser *et al, 2009*). Through in situ hybridization, the known core odontogenic genes were screened against taste buds at analogous developmental stages (i.e. initiation, morphogenesis, differentiation) to identify genetic similarities. Based on observations of the structural similarity of tooth and taste bud histology in early development, co-localization within the oropharyngeal cavity, common tissue origin, and shared gene expression, it was hypothesized that the developmental programs of teeth and taste buds were governed using similar gene networks.

CHAPTER 2

METHODS

Overview

In order to acquire histological gene expression data for developing taste buds and teeth in cichlid embryos, a lengthy series of techniques is required. Experimental methods start from reproducing adults in aquariums and end with examining the individual cells of ultrathin embryonic sections under a microscope. Fish broods must be taken from mouth-brooding females and preserved using tissue fixative. Embryos must then be prepared for in situ hybridization whereby synthesized RNA probes bind to complementary sequences of target mRNA strands. In doing so, highly specific gene expression patterns are visualized throughout the embryo body. After whole mount analyses are completed, embryos with proper gene expression signals are embedded into gelatin-albumin blocks. The blocks are then cut into ultrathin sections which are viewed under high magnification microscopes. At this stage, pinpointing gene expression patterns among individual cells is possible, thereby allowing histological genetic analysis of tooth and taste bud development possible as well.

Cichlid Aquaculture

Several species of African cichlids from Lake Malawi were maintained in aquariums within a carefully monitored environment. Aquarium water was managed using a recirculating system that maintained water temperature at 28˚C while rotating 12 hour night and day cycles. Fish were either separated into different tanks according to species or shared one tank with fish from other species. Species used in this study include *Copadichromis conophorus*, *Cynotilapia afra*, *Metriaclima zebra albino,* and *Labeotropheus fuelleborni*. Broods from mouth-brooding females were either separated

into separate culture tanks or fixed once the desired embryonic stage was reached. Embryonic stage was determined according to days post-fertilization (dpf).

Light Microscopy

Whole mount specimens were viewed under a Leica MZ16 stereo microscope. Embryos were placed over agarose gel in Petri dishes and suspended in either PBS or glycerol. Histological sections were viewed through a Leica DM2500 compound microscope. Sectioned embryos were mounted on clear glass slides which adhered to the glass cover slips using small drops of slightly diluted glycerol. An attachable LFC digital camera photographed the images that were scaled appropriately using Leica Microsystems software and analyzed using Adobe Photoshop®.

RNA Probe Synthesis

Probe Design

Primers were designed by matching gene sequence data from relevant cichlid species (Loh et al, 2008) with the results from NCBI searches for equivalent genes. After another NCBI search using BLAST verified a precise match with the genes of interest, primers were created using Primer3. Typically, primers exhibited a length of approximately 800 base pairs to ensure high specificity and appropriate binding affinity.

Polymerase Chain Reaction (PCR)

Once properly constructed, desired genes were amplified using the polymerase chain reaction. Heat controlled cycles of annealing and dissociation were run to amplify the target gene. Once the lengths of the isolated fragments were estimated appropriately

against a control ladder in a gel electrophoresis, the products were cleaned and subjected to another round of polymerase chain reaction.

Colony PCR

The PCR products were amplified by transforming them into competent *Escherichia coli* cell and allowing amplification on agar plates. Ampicillin on the agar plates ensured that successfully transformed colonies were identifiable by virtue of a characteristic white color. Plasmids transformed in colonies were amplified with PCR and analyzed for purity using gel electrophoresis.

DNA Sequencing

Plasmid DNA was sequenced for accuracy and analyzed for percent confidence using Sequencher®. Highly purified sequences were verified using the NCBI BLAST tool (www.ncbi.nih.nlm.gov) in order to confirm the identity of the sequenced target gene.

Synthesis of RNA Probes

High purity sequences of the target DNA were linearized using specific restriction enzymes and then cleaned using a phenol:chloroform solution. The linearized DNA plasmids were added to transcription buffer, digoxenin-RNA labeling mix (Roche), and RNase inhibitor (Roche). After incubation for two hours, the mixture was centrifuged vigorously to create a pellet of DNA that was cleaned, dried, and finally resuspended in DEPC-H_20. The suspended DNA was added to a hybridization solution containing formamide, buffer, citric acid, tween-20, DEPC-H_20, tRNA, and heparin.

RNA In Situ Hybridization

The *in situ* hybridization protocol used in this study follows that of a previous publication (Fraser et al, 2004). Embryos were set briefly in PBS before being fixed in 4% PFA and then dehydrated in a graded methanol series. After being rehydrated back into PBS, embryos were subjected partial tissue digestion using a buffer solution containing proteinase K. Digested embryos were then refixed in 4% PFA and rinsed. Embryos were then suspended in a prehybridization solution and placed over a 70˚C water bath. While remaining at constant temperature, embryos were incubated overnight in hybridization solution containing a specific digoxigenin-labeled mRNA probe. The hybridization solution was then replaced with fresh prehybridization solution at room temperature and rinsed into a graded sodium-chloride sodium citrate buffer solution. The embryos were then washed in MABT before being incubated in blocking solution on a gentle rocker. The blocking solution was replaced by fresh blocking solution containing AP anti-digoxenin fragments within which the embryos were incubated overnight at 4˚C. The embryos were then washed several times in TST solution. Next, the embryos were rinsed twice in NTMT solution before NBT/BCIP diluted in NTMT was added to the embryos. Once the desired expression signal of the embryos was reached, the color reaction was stopped using PBST. The embryos were then post-fixed in 4% PFA and rinsed in PBS.

TISSUE SECTIONING

Embryos that exhibited desirable gene expression after in situ hybridization were prepared for fine sectioning for histological analysis. Embryos were implanted into a small well containing a viscous gelatin-albumin (from chick egg) solution and oriented according to the desired plane of the embryo cross-section. Diluted glutaraldehyde was added to harden the gelatin-albumin solution before being stored overnight at 4˚C in a highly humid environment. Next, the hardened blocks were postfixed in 4% PFA and

then washed in PBS. Appropriate trimmed blocks were then carefully superglued onto an attachable platform on a Leica Microsystems VT1000 Vibratome. Once mounted and submerged in PBS, blocks were sliced into 15-25µm sections that were carefully positioned onto clear glass slides and prepared for light microscopy.

CHAPTER 3

RESULTS

A list of the spatiotemporal patterns of gene expression in taste buds is organized into Table 1. Odontogenic genes are categorized into their location of expression among tissue layers: epithelium, mesenchyme, or both. Genes in taste buds, however, are shown as having positive or negative expression. The arrangement of Table 1 is such that it clearly shows which dental genes are also employed in developing taste buds and that it elucidates the observation that odontogenic mesenchymal genes are too expressed in the strictly epithelial development of taste buds. Further, genes with positive expression are categorized into their location of expression within the taste bud proper: apical, basal, or whole taste bud.

Cichlid odontogenic genes found to be involved in taste bud formation include β-cat, Bmp4, Fgf3, Fgf10, Notch2, Pax9, Pitx2, Ptc2, Tbx1, and Shh. Most of these genes have known roles in the vertebrate core dental network of genes including β-cat, Bmp4, Fgf3, Notch2, Pitx2, and Shh (Fraser et al, 2009).

Gene	Teeth	Taste Bud Initiation	Taste Bud Morphogenesis	Taste Bud Differentiation
b-cat	E + M	Whole	Whole	Basal
bmp2	E + M	X	X	X
bmp4	E + M	Basal	X	X
eda	M	X	X	-
edar	E	X	X	-
fgf3	E	Apical	Apical	Apical
fgf10	M	Whole	Whole	-
notch2	E	X	Whole	Whole
pax9	M	Whole	Whole	Whole
pitx2	E	X	X	Basal
ptc2	E	-	Apical	Whole
shh	E	Basal	Basal	Basal
tbx1	E	Whole	Whole	Whole
wnt10	E	Whole	Whole	-

Table 1. Patterns of Gene Expression in Teeth and Taste Buds. E, epithelium. M, mesenchyme. X, no expression. -, not determined.

Figure 3a and 3b demonstrates the variable spatial patterns of gene expression within a developing taste bud. Genes that exhibited expression throughout the taste bud include β-cat, Fgf10, Notch2, Pax9, Tbx1, and Wnt10; however, β-cat became restricted to the basal region during taste bud differentiation and Notch2 was not expressed until morphogenesis. Interestingly, the only apically expressed genes were Fgf3 and then Ptc2 during the morphogenesis stage. Genes with expression confined to the basal region of taste buds include Bmp4, Pitx2, and Shh; however, Bmp4 was no longer expressed after taste bud initiation and Pitx2 only began showing expression during the differentiation stage. Additionally, another odontogenic gene that demonstrated positive expression in developing taste buds was Dec1 during the late differentiation stage (Boudjelal et al, 1997). It is notable that Fgf10 is also part of the core dental network and plays a strictly mesenchymal role in tooth development (Fraser et al, 2009).

Genes with known involvement in odontogenesis never exhibited expression during taste bud development such as Bmp2. Although it was not determined for the differentiation stage, Eda and Edar were not expressed in taste buds. It is notable as well that Eda and its receptor protein, Edar, are both expressed in initiated teeth but did not exhibit expression in initiated taste buds.

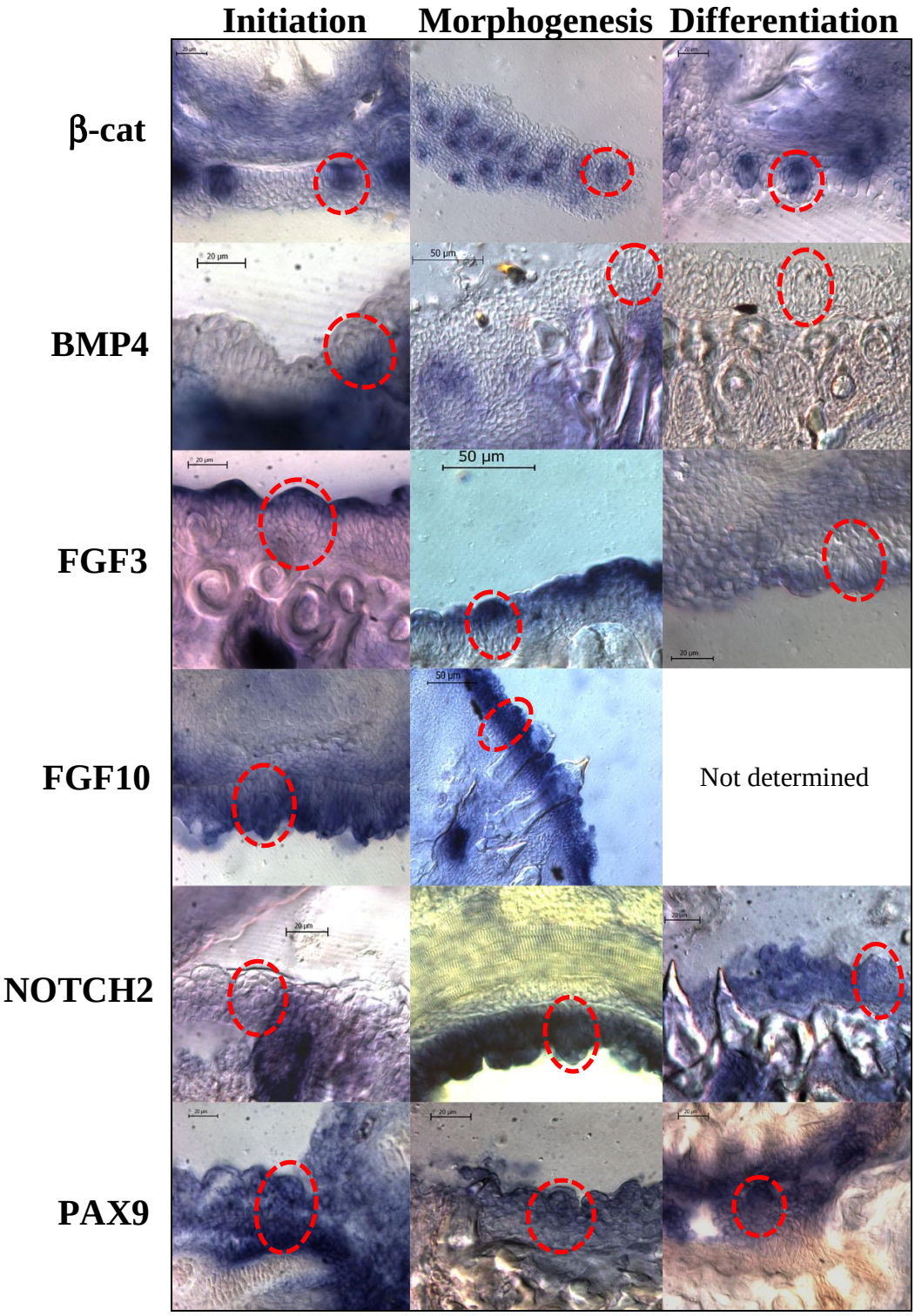

Figure 3a. Gene Expression in Taste Buds at Progressive Developmental Stages. Embryos from 8-12dpf were classified into the Initiation stage for taste buds, from 14-18dpf into the morphogenesis stage, and from 24-28dpf into the Differentiation stage. The dashed red circles encapsulate a characteristic taste bud. The images shown represent a sample of many similar images taken.

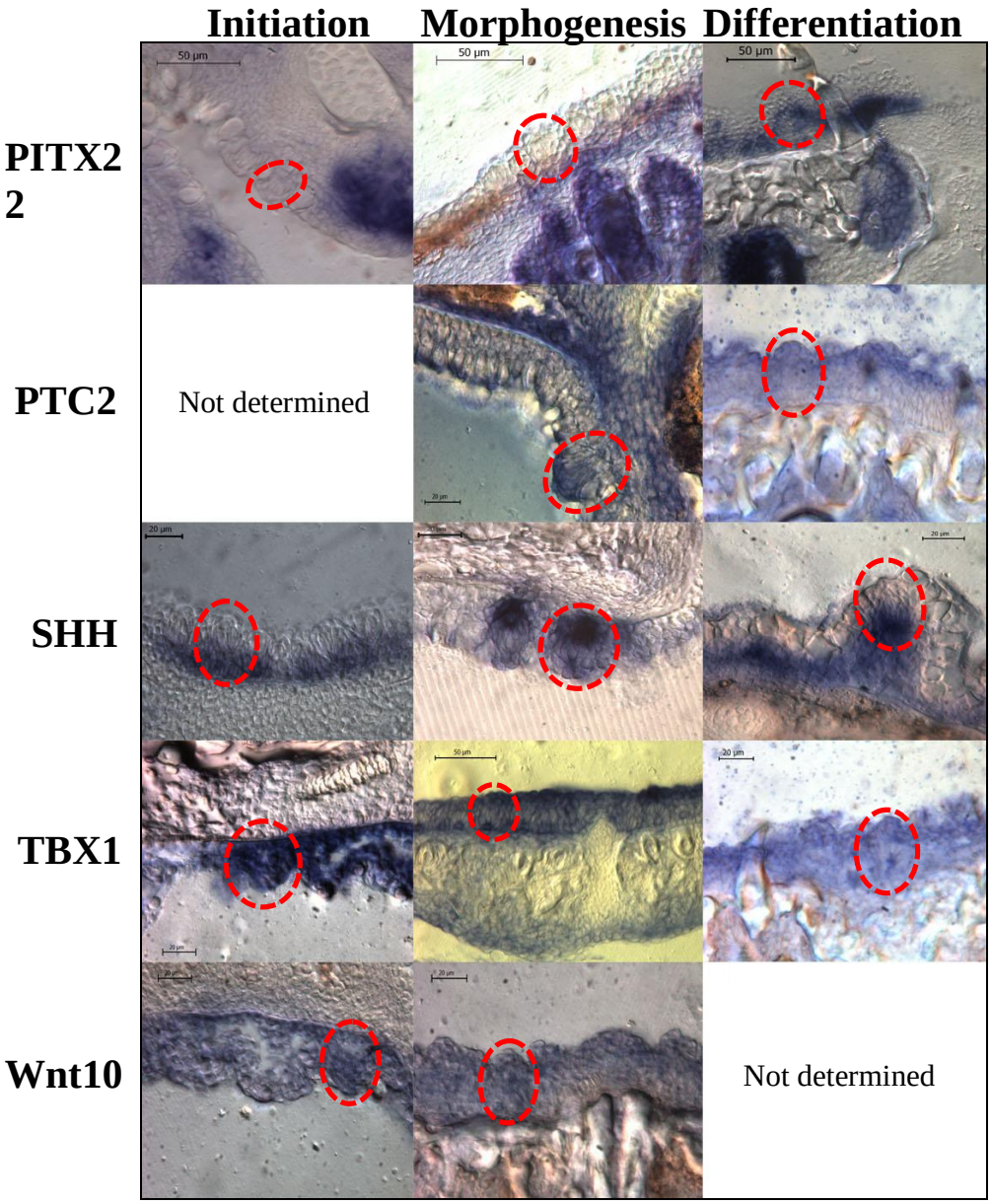

Figure 3b. Gene Expression in Taste Buds at Progressive Developmental Stages. Embryos from 8-12dpf were classified into the Initiation stage for taste buds, from 14-18dpf into the morphogenesis stage, and from 24-28dpf into the Differentiation stage. The dashed red circles encapsulate a characteristic taste bud. The images shown represent a sample of many similar images taken.

CHAPTER 4

DISCUSSION

The aim of this research was to generate evidence that would support the theory that teeth and taste buds share a co-evolutionary history. This study sought to accomplish that by coupling the developmental programs of the two epithelium-derived structures. Using comparative morphology and molecular biology in the context of development, this study shows that the gene networks governing development of teeth and taste buds are similar. Furthermore, resemblances of genetic networks are too observable at analogous stages of tooth and taste bud development: initiation, morphogenesis, and differentiation. Figure 4 illustrates the coupling of the developmental programs of teeth and taste buds at analogous stages of development.

At the initiation stage of the teeth and taste buds, commonly expressed genes were β-cat, Bmp4, Fgf3, Fgf10, Pax9, and Shh. This stage is unique in that both teeth and taste buds appear much alike as thickened placodes within the epithelial tissue layer. Also unique to this stage is the common lack of signaling of the epithelium with the subjacent mesenchyme. An important difference in gene expression at this stage is the lack of Edar expression in taste buds. This gene codes for the receptor for the Eda protein signal originating in the mesenchyme. Zebrafish with a mutant Edar genotype have significantly abnormal tooth development that results in phenotypes much like hypohidrotic ectodermal dysplasia in mammals with the same mutation (Harris et al, 2008). It is inferable then that a staple early difference between teeth and taste buds is that taste buds lack Edar, rendering them incapable of receiving early mesenchymal Eda signals. Together with co-localization and common tissue origins, the close structural similarity and shared expression of β-cat, Bmp4, Fgf3, Fgf10, Pax9, and Shh during initiation suggest evolutionary relatedness of teeth and taste buds and hints towards a possible homologous relationship.

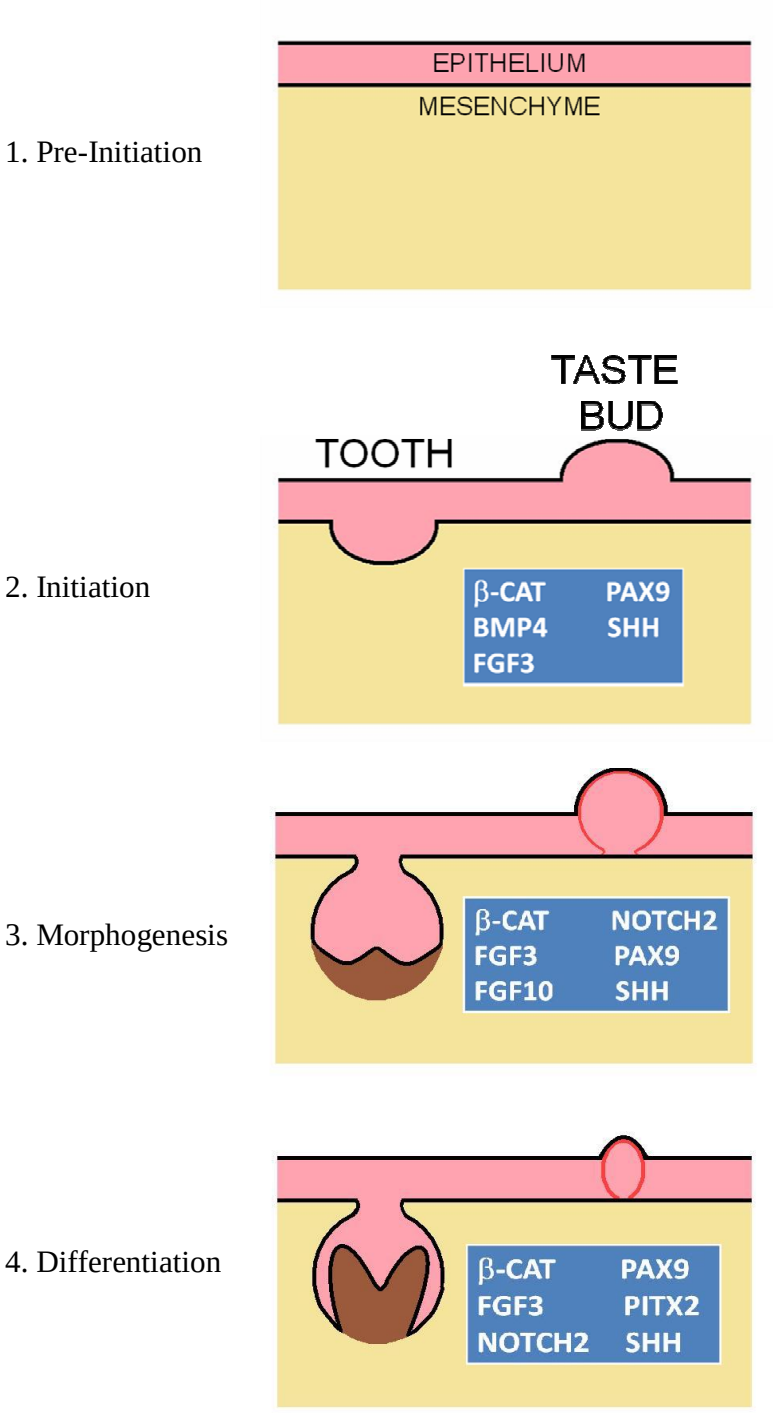

Figure 4. Schematic of the Coupled Developmental Programs of Teeth and Taste Buds. The development of a tooth is outlined on left and that of a taste bud on the right. Genes listed in the blue boxes are genes of the core dental network that are expressed in taste buds as well.

In the morphogenesis stage, teeth and taste buds share expression of β-cat, Fgf3, Fgf10, Notch2, Pax9, and Phh. It is during this stage that the fundamental difference in the developmental programs between teeth and taste buds is seen. The tooth bud begins exchanging signals with the underlying mesenchyme while the taste bud remains independent from communication with the mesenchyme. Perhaps the lack of odontogenic epithelial Bmp2, Bmp4, and Pitx2 expression in morphogenetic taste buds can partially account for their inability to communicate with mesechymal cells at this stage. Ptc2 exhibits expression in this stage possibly as a result of Shh and Bmp4 signals activating transcription of Ptc2. Apart from Bmp2, Bmp4, and Pitx2, however, gene expression of the two structures during morphogenesis is very similar, suggesting regulatory gene conservation and thus likely phylogenetic relatedness.

During the differentiation stage of teeth and taste buds, commonly expressed genes were β-cat, Fgf3, Notch2, Pax9, Pitx2 and Shh. Cells beginning to differentiate into their respective terminal cell types mark this stage of development. Until this stage, Pitx2 was never expressed in the taste bud. It is plausible that this gene has been activated for the purposes of cell differentiation in the basal region of taste buds, perhaps playing a role in innervation.

Surprisingly, the stage with the least amount of shared gene expression was the initiation stage but the greatest amount of co-expressed genes was during the differentiation stage. This observation was not expected because teeth and taste buds are morphologically most similar during the initiation stage and least similar during differentiation. Moreover, based on histological and regional similarities of teeth and taste buds during epithelium initiation, it is logical to expect an equivalently high degree of similarity in gene expression (Sanetra et al, 2005). However, this observation may only exist on account of the limited number of genes used for screening in this study and may not be observed if more of the hundreds of odontogenic genes were tested for expression in taste buds. Equally possible, the gene networks that have been activated for

the morphogenesis and differentiation stages in tooth and taste bud development bear more resemblance through conservation than the earlier gene networks that do the activating.

As with most observations in evolutionary biology, the co-evolution of teeth and taste buds cannot be proven. This theory is only capable of being supported with sound evidence that explains observed trends. There exist several ways to generate evidence for this relationship, but this study focuses only on common patterns of gene network expression domains. Of course, it is important to point out that the domains of gene expression do not singularly reflect common descent. A stronger case would be made by showing synexpression groups or gene regulatory networks (Nielsen et al, 2002). Nevertheless, this study has shown that the gene networks that regulate tooth morphogenesis are too conserved in tooth development including the Bmp, Fgf, Wnt, and Shh genes. This observation is likely a result of a co-option of not just a few genes, but rather of an entire suite of regulatory genes that has probably created new regulatory linkages with signaling pathways (Pires-daSilva et al, 2003). It has been recorded several times in many studies that evolutionary novelties arise more often through genetic combinatorial processes such as changes in gene regulation rather than adaptive evolutionary processes to DNA coding such as creation of a novel gene (Sanetra et al, 2005). In other words, evolution more likely produced the significant morphologic difference between teeth and taste buds by using the same genes in a different way through variation in gene regulation than by creating an entirely new group of genes. Other studies have shown that evolutionary changes to gene regulatory networks since divergence are limited sharply to specific *cis*-regulatory elements, whereas others have persisted unaltered (Hinman et al, 2003). This evolutionary developmental theme may well influence future studies seeking to show co-evolution of teeth and taste buds.

CHAPTER 5

CONCLUSION

This study provides evidence supporting the theory that teeth and taste buds in vertebrates share a co-evolutionary history. Future studies will be required, however, before this theory is supported conclusively. Although several odontogenic genes were screened against developing taste buds in this study, there exist many more genes that play crucial roles in the development of the two structures. Therefore, a much larger network of genes in the developmental programs of the teeth and taste buds should be thoroughly characterized for each. Using this data, a deeper comparison of the two networks can be made to reveal further correlations in gene expression during analogous stages of development. Additionally, studies in functional genomics may reveal equivalent roles of specific co-expressed genes or groups of genes in the development of early teeth and taste buds such as basal innervation or patterning within the oropharynx. Also, future studies might seek to uncover a correlation between the number of teeth and the number of taste buds within the same regions of the oropharyngeal cavity, either in developing embryos or fully grown adults.

The research presented in this study supports the hypothesis that teeth and taste buds are governed by similar gene networks. By virtue of comparative morphology, developmental biology, and molecular biology, this study provides evidence suggesting a homology of teeth and taste buds in the light of evolutionary biology. Based on their shared stages of development, colocalization within the oropharynx, remarkable structural similarity during initiation, and deployment of conserved regulatory gene networks, vertebrate teeth and taste buds likely share a co-evolutionary history.

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